of antioxidants added to a saline at physiologic concentrations.[16] Studies have shown that $TRAP_{Unid}$ seems to react sharply in cases of oxidative stress. In acute infection the amount of classical antioxidants remained unchanged, whereas the amount of unidentified antioxidants declined significantly.[14] Immobilized patients with a decreased basic metabolism and free radical production showed a significant decrease in TRAP primarily because of a decline in the concentration of unknown antioxidants, which may be in connection with the basic metabolic rate.[15] Other results from our laboratory also support the idea that $TRAP_{Unid}$ is composed of actions of endogenous products and may be related to basic metabolism; in addition in heavy exercise, a significant increase in $TRAP_{Unid}$ can be detected.[17–20]

When looking for the possible candidate molecules responsible for $TRAP_{Unid}$, several conditions must be fulfilled. Such a compound(s) must be present in plasma in sufficient concentrations and must be capable of scavenging peroxyl radicals. $TRAP_{Unid}$ has been proposed to be composed of actions of endogenous compounds, such as albumin, glucose, cholesterol, steroids, and melatonin, but antioxidants from nutritional origin such as flavonoids or benzylisoquinoline alkaloids cannot yet be totally ruled out.[31] Finally, the nature and role of $TRAP_{Unid}$ are the focal concerns of further investigations, and the wide variety of candidates ensure that it will be an interesting challenge.

In conclusion, chemiluminescence-enhanced TRAP has revealed important information in evaluating the antioxidant status of human plasma. Plasma antioxidant defenses seem to respond to needs related to the basic metabolic rate and to the challenges caused by physiological or pathological stress. According to our studies, an important, possibly endogenous, antioxidant remains to be identified. However, the total antioxidative potential cannot be evaluated reliably by measuring only TRAP because of its dependence on uric acid. TRAP combined with its main components is likely to give more valid information in determining the total antioxidant status.

[31] R. J. Reiter, D. Melchiorri, E. Sewerynec, B. Poeggler, L. Barlow-Walden, J. Chuang *et al.*, *J. Pineal Res.* **18,** 1 (1995).
[32] D. D. M. Wayner, G. W. Burton, K. U. Ingold, L. R. C. Barclay, and S. J. Locke, *Biochim. Biophys. Acta* **924,** 408 (1987).

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Acknowledgments

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[2] Ferric Reducing/Antioxidant Power Assay: Direct Measure of Total Antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration

By Iris F. F. Benzie and J. J. Strain

Introduction

The ferric reducing/antioxidant power (FRAP) assay[1,2] is a recently developed, direct test of "total antioxidant power." Other tests of total antioxidant power used to date are indirect methods[3–8] that measure the ability of antioxidants in the sample to inhibit the oxidative effects of reactive species purposefully generated in the reaction mixture. In inhibition assays, antioxidant action induces a lag phase; exhaustion of antioxidant power is denoted by a change in signal, such as rate of oxygen utilization, fluorescence, or chemiluminescence. Measurement of these signals requires specialized equipment, and such tests can be time-consuming, technically demanding, and may lack sensitivity.[9]

In contrast to other tests of total antioxidant power, the FRAP assay is simple, speedy, inexpensive, and robust. The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an

[1] I. F. F. Benzie and J. J. Strain, U.S. Patent Pending (1997).
[2] I. F. F. Benzie and J. J. Strain, *Anal. Biochem.* **239,** 70 (1996).
[3] D. D. M. Wayner, G. W. Burton, K. U. Ingold, L. R. C. Barclay, and S. J. Locke, *Biochim. Biophys. Acta* **924,** 408 (1987).
[4] T. P. Whitehead, G. H. G. Thorpe, and S. R. J. Maxwell. *Anal. Chim. Acta* **266,** 265 (1992).
[5] N. J. Miller and C. A. Rice-Evans, *Redox Rep.* **2,** 161 (1996).
[6] G. Cao, H. M. Alessio, and R. G. Cutler, *Free Radic. Biol. Med.* **14,** 303 (1993).
[7] E. Lissi, M. Salim-Hanna, C. Pascual, and M. D. Del Castillo, *Free Radic. Biol. Med.* **18,** 153 (1995).
[8] A. Ghiselli, M. Serafini, G. Maiani, E. Azzini, and A. Ferro-Luzzi, *Free Radic. Biol. Med.* **18,** 29 (1995).
[9] D. Schofield and J. M. Braganza, *Clin. Chem.* **42,** 1712 (1996).

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easily reduced oxidant present in stoichiometric excess. Unlike the many indirect radical scavenging tests designed to measure total antioxidant power, the FRAP assay does not use a lag phase type of measurement. In the FRAP assay, sample pretreatment is not required, stoichiometric factors are constant, linearity is maintained over a wide range, reproducibility is excellent, and sensitivity is high.[2] The FRAP assay does not need highly specialized equipment or skills, or critical control of timing and reaction conditions. The FRAP assay can be performed using automated, semiautomated, and manual versions, and, in a modified version known as the ferric reducing/antioxidant power and ascorbic acid concentration (FRASC[10]) assay, supplies three indices of antioxidant status—the total reducing (antioxidant) power, the absolute concentration of ascorbic acid, and the relative contribution of ascorbic acid to the total antioxidant power of the sample—virtually simultaneously.

Concept of FRAP Assay

A biological antioxidant has been defined as "any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate."[11] However, unless an antioxidant prevents the generation of an oxidizing species, for example, by metal chelation or enzyme-catalyzed removal of a potential oxidant, a redox reaction still generally occurs, even in the presence of an antioxidant. The difference is that the oxidizing species reacts with the antioxidant instead of the "substrate," i.e., the antioxidant reduces the oxidant. In simple terms then, electron-donating antioxidants can be described as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reactive species is reduced while another is oxidized. In this context, therefore, "total antioxidant power" may be referred to analogously as total reducing power.

Principle of FRAP Assay

At low pH, reduction of a ferric tripyridyltriazine (Fe^{III}-TPTZ) complex to the ferrous form, which has an intense blue color, can be monitored by measuring the change in absorption at 593 nm. The reaction is nonspecific, in that any half-reaction that has a lower redox potential, under reaction conditions, than that of the ferric/ferrous half-reaction will drive the ferric (Fe^{III}) to ferrous (Fe^{II}) reaction. The change in absorbance, therefore, is

[10] I. F. F. Benzie and J. J. Strain *Redox Rep.* **3**, 233 (1997).
[11] B. Halliwell and J. M. C. Gutteridge, *Free Radic. Biol. Med.* **18**, 125 (1995).

directly related to the combined or "total" reducing power of the electron-donating antioxidants present in the reaction mixture.

Materials and Methods

Reagent Preparation

Mix 300 m*M* acetate buffer, pH 3.6 [3.1 g sodium acetate trihydrate (Riedel-de Haen, Germany), plus 16 ml glacial acetic acid (BDH Laboratory Supplies, England) made up to 1 liter with distilled water]; 10 m*M* TPTZ (2,4,6-tripyridyl-*s*-triazine, Fluka Chemicals, Switzerland) in 40 m*M* HCl (BDH); and 20 m*M* $FeCl_3 \cdot 6H_2O$ (BDH) in the ratio of 10:1:1 to give the working FRAP reagent. Prepare working reagent fresh as required.

The following antioxidants are used to evaluate the FRAP assay. Solid L-(+)-ascorbic acid extra pure crystals (Merck, Germany); uric acid, solid (BDH); albumin, solid (bovine serum albumin, fraction V, Sigma Chemical Co., St. Louis, MO); bilirubin calibrator solution (Sigma); and Trolox, the water-soluble analog of α-tocopherol (Aldrich Chemical Co., Milwaukee, WI), are used in aqueous solutions of known concentrations. DL-α-Tocopherol (Merck) is diluted in ethanol (Merck) to give required concentrations.

Standards and Controls

Aqueous solutions of known Fe(II) concentration ($FeSO_4 \cdot 7H_2O$; Riedel-de Haen) and/or freshly prepared aqueous solutions of a pure antioxidant, such as ascorbic acid (extra pure crystals, Sigma), are used for calibration of the FRAP assay. Reaction of Fe(II) represents a one electron exchange reaction and is taken as unity, i.e., the blank corrected signal given by 100 μM solution of Fe(II) is equivalent to a FRAP value of 100 μM. Typical Fe(II) standard concentrations used in our laboratories are in the range of 100–1000 μM. Ascorbic acid has a constant stoichiometric factor of 2.0 in the FRAP assay, i.e., direct reaction of Fe(II) gives a change in absorbance half that of an equivalent molar concentration of ascorbic acid (see later). An ascorbic acid standard of 1000 μM, therefore, is equivalent to 2000 μM of antioxidant power as FRAP.

Pooled, aged plasma stored at −70° and thawed overnight at 4°, or frozen, aliquoted commercially available QC serum can be used to monitor precision. In addition, freshly prepared solutions of pure antioxidants, such as uric acid, Trolox, and ascorbic acid, in known concentration in aged plasma, commercially available QC serum, or in aqueous solution can be used to monitor accuracy and precision. For ease of use and reliability,

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aqueous ascorbic acid solutions at 100, 250, 500, and 1000 μM (equivalent to 200, 500, 1000, and 2000 μM FRAP) prepared fresh daily and aged QC serum freshly spiked with ascorbic acid are recommended as quality control samples. These should be run in parallel with test samples to actively monitor the performance of the test and to ensure comparability with previous results.

Samples

The FRAP assay can be performed on a wide range of complex biological fluids, including plasma, serum, saliva, tears, urine, cerebrospinal fluid, exudates, transudates, and aqueous and ethanolic extracts of drugs, foods, and plants, as well as on simple and heterogeneous solutions of pure antioxidants. If, using the reaction conditions described, the FRAP value of a sample is >3000 μM, it is recommended that the sample be diluted in water or ethanol, as appropriate, and the test repeated, with the additional dilution factor allowed for during the final calculation of the FRAP value.

Procedure for Automated FRAP Assay

The FRAP assay can be performed using any type of automated analyzer that permits blank corrected readings at 593 nm to be taken at predetermined intervals after sample–reagent mixing. In our laboratories the Cobas Fara centrifugal analyzer is used, and the user-defined test program is presented in Table I. The 0- to 4-min reaction time window is used for data capture for plasma total antioxidant power. Absorbance change is translated into a FRAP value (in μM) by relating the $\Delta A_{593\,nm}$ of test sample to that of a standard solution of known FRAP value [e.g., 1000 μM Fe(II)] shown in Eq. (1):

$$\frac{\text{0- to 4-min } \Delta A_{593\,nm} \text{ test sample}}{\text{0- to 4-min } \Delta A_{593\,nm} \text{ standard}} \times \text{FRAP value of standard } (\mu M) \quad (1)$$

This additional step can be added easily to the analyzer test program if desired in order to give a direct printout of FRAP values in μM.

Procedure for Manual FRAP Assay

To perform the FRAP assay manually, the same working reagent, standards, controls, and test samples are used; reagent and sample volumes are simply increased pro rata to give a volume large enough for manual handling/transfer of reaction mixtures. For example, 3.0 ml of working FRAP reagent is mixed with 100 μl test sample or standard in a test tube; this is vortex mixed, and the absorbance at 593 nm is read against a reagent blank at a predetermined time after sample–reagent mixing. If the test is performed at 37°, the 0- to 4-min reaction time window is used for plasma; if performed at room temperature, a 0- to 6-min reaction time window is preferable as the reaction of uric acid is slightly slower at lower temperatures. The calculation of results is the same as for the automated method.

TABLE I
COBAS FARA TEST PROGRAM FOR AUTOMATED FRAP ASSAY[a]

Measurement mode	Abs
Reaction mode	R1-I-S-A
Reagent blank	reag/dil
Wavelength	593 nm
Temperature	37°
R1	300 μl
M1	1.0 sec
Sample volume	10 μl
Diluent name	H_2O
Volume	30 μl
Readings	
First	0.5 sec
Number	17
Interval	15 sec
Reaction direction	Increase
Number of steps	1
Calculation	End point
First	M1
Last	17 (i.e., 4 min) for FRAP 5 (i.e., 1 min) for ascorbic acid

[a] Reproduced with permission from I. F. F. Benzie and J. J. Strain, *Redox Rep.* 3, 233 (1997).

Results

Results are presented for pure solutions of ascorbic acid, α-tocopherol, and uric acid; for more complex aqueous mixtures of pure antioxidants; and for fresh, fasting plasma from apparently healthy adults. Preliminary data on FRAP values of selected beverages are also given.

FRAP Reaction Characteristics of Pure Antioxidants

Figure 1 shows the post sample–reagent mixing change in absorbance at 593 nm for equimolar solutions of different antioxidants compared to the monitored absorbance of working FRAP reagent only using the automated FRAP assay. Ascorbic acid and α-tocopherol react very quickly, with a

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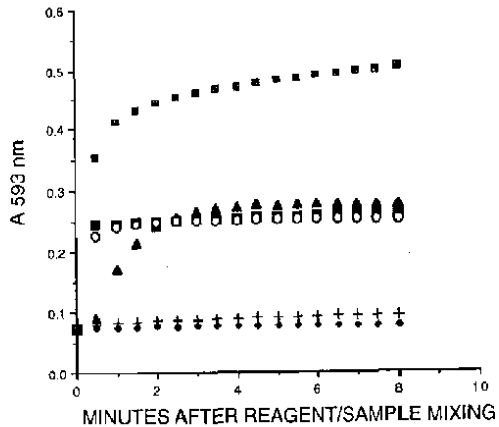

FIG. 1. FRAP reaction kinetics with individual antioxidants; rate of increase in absorbance at 593 nm for 100 μM solutions of bilirubin (⊞), ascorbic acid (■), uric acid (▲), α-tocopherol (○), albumin (+), and reagent alone (◆). Reproduced with permission from I. F. F. Benzie and J. J. Strain, *Anal. Biochem.* **239,** 70 (1996).

plateau reached within a few seconds. The reaction of uric acid reaches an end point after 3 min, but the reaction with bilirubin and with albumin does not appear to have reached an end point at 6 min. The reaction of bilirubin is fast in the first few seconds, however, and the continued slow increase in absorbance at 593 nm is due to the albumin content of the bilirubin preparation used.[2]

Figure 2 shows the dose–response characteristics of individual antioxidants in the FRAP assay. Whereas different antioxidant "efficiencies" can be seen, the dose–response line of each individual antioxidant tested is linear, showing that antioxidant efficiency is not concentration dependent. The relative activity is 2.0, i.e., direct reaction of Fe(II) gave a change in absorbance half that of an equivalent molar concentration, for Trolox, α-tocopherol, ascorbic acid, and uric acid. After correcting for albumin content, the stoichiometric factor of bilirubin in the FRAP assay is estimated to be 4.0. The activity of albumin in the FRAP assay is very low.[2] This is an advantage of FRAP, as the contribution of and changes in nonprotein antioxidants can be measured without the muffling and possibly misleading effect of the dominating contribution of protein seen in other methods of total antioxidant power.[3–6]

When the FRAP assay is performed with plasma and with aqueous solutions of antioxidants but with no Fe(III) added to the working reagent, no color develops. This indicates that there is no detectable free Fe(II) in

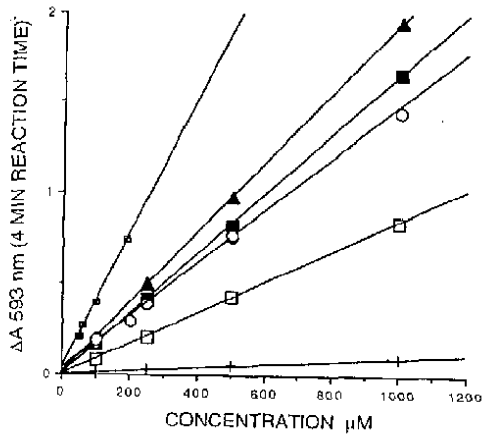

FIG. 2. Linearity of FRAP: dose–response lines for solutions of bilirubin preparation (+), uric acid (▲), ascorbic acid (■), α-tocopherol and Trolox (○), Fe(II) (□), and albumin (+). Reproduced with permission from I. F. F. Benzie and J. J. Strain, *Anal. Biochem.* **239,** 70 (1996).

plasma and that there is no detectable agent in normal plasma that reacts directly with TPTZ to form the blue ferrous-TPTZ chromogen. Monitoring the FRAP working reagent with no sample addition shows that no color develops (Fig. 1), indicating negligible spontaneous Fe(III) reduction in the absence of added reductants (antioxidants).

There is no apparent interaction between antioxidants in the FRAP assay. When known amounts of individual antioxidants were mixed and the FRAP value measured, there was good recovery of antioxidant power (91–112%), and good agreement was seen between anticipated and measured FRAP values ($r = 0.990$; $P < 0.001$) when known amounts of individual antioxidants were added to plasma and to water (Fig. 3). In addition, the FRAP dose–response relationship was the same, i.e., parallel lines were obtained, when uric acid solutions of different concentrations were tested with and without the presence of 100 μM ascorbic acid and when different concentrations of ascorbic acid were tested with and without 200 μM uric acid (Fig. 4).

Precision and Sensitivity of FRAP Assay

Precision is excellent: within-run coefficients of variation (CVs) are <1.0% at FRAP values of 100, 200, and 900 μM and the between-run CV is <3.0% at 960 μM. The limit of detection of the FRAP assay is <2 μM reducing/antioxidant power.

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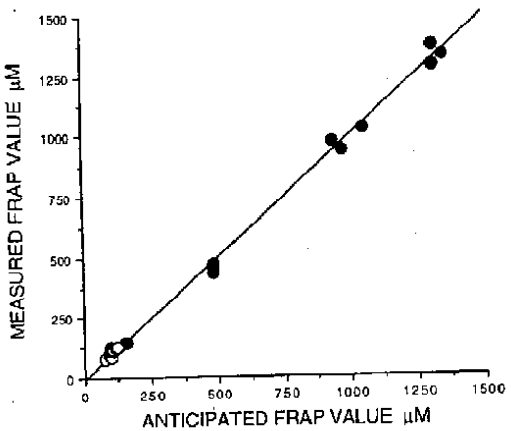

FIG. 3. Relationship between anticipated (calculated) FRAP values and measured FRAP values when known amounts of pure antioxidants were added to plasma (●) and water (○); $r = 0.99$, $P < 0.001$. Reproduced with permission from I. F. F. Benzie and J. J. Strain, *Anal. Biochem.* **239,** 70 (1996).

FRAP Values Obtained on Biological Fluids

The mean (SD) FRAP value of fresh, fasting plasma from 68 apparently healthy, consenting adults was 1035 (226) μM (range 638–1634 μM). There was a significant correlation between FRAP values and plasma uric acid

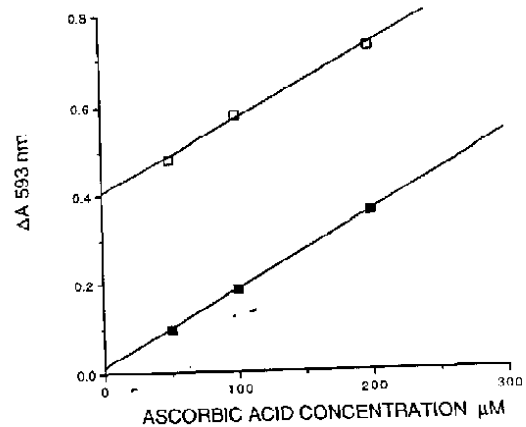

FIG. 4. Study of interaction between uric acid and ascorbic acid in the FRAP assay: the FRAP dose–response relationship of ascorbic acid in water (■) and in an aqueous 200 μM uric acid solution (□).

TABLE II
RELATIVE ANTIOXIDANT ACTIVITY OF INDIVIDUAL ANTIOXIDANTS AND THEIR ESTIMATED CONTRIBUTION TO FRAP VALUE OF FRESH FASTING PLASMA[a]

Plasma antioxidant	Relative activity "antioxidant efficiency" (measured range)	Expected fasting plasma concentration (μM)	Estimated contribution to total FRAP (%)
Ascorbic acid	2.0 (1.9–2.1)	30–100	15
α-Tocopherol	2.0 (1.7–2.1)	15–40	5
Uric acid	2.0 (2.0–2.4)	150–450	60
Bilirubin	4.0 (4.2–4.6)	<20	5
Protein	0.10 (0.1–0.15)	800–1,100	10
Others	—	—	5

[a] Modified and reproduced with permission from I. F. F. Benzie and J. J. Strain, *Anal. Biochem.* **239,** 70 (1996).

concentrations ($r = 0.913$, $P < 0.001$; $n = 68$), with uric acid contributing around 60% of the total antioxidant power of plasma. However, as stoichiometric factors are constant in the FRAP assay, it is a simple matter to subtract the contribution of uric acid to "total" antioxidant power; the measured concentration (in μM) of uric acid multiplied by its stoichiometric factor gives the μM antioxidant power of the sample due to uric acid. Subtracting this from the FRAP value of the sample gives the nonuric acid FRAP value,[10,12] which may offer a more sensitive index of antioxidant status in uric acid-rich fluids such as plasma. The relative contribution to total reducing/antioxidant power of other major antioxidants in plasma has also been calculated (Table II). Table III shows the representative range of FRAP values found in teas, wines, and orange juices, plant-based dietary agents that are rich in polyphenolic antioxidant compounds.

FRASC–FRAP and Ascorbic Acid Measurements in One Combined Assay

The measurement of ascorbic acid can be problematical due to its instability in plasma, and the specific measurement of ascorbic acid has generally required pretreatment of plasma to stabilize its ascorbic acid content, followed by high-performance liquid chromatography (HPLC) analysis.[13,14] The FRASC assay is a modification of the FRAP assay that

[12] I. F. F. Benzie and J. J. Strain, *Redox Rep.* **2,** 231 (1996).
[13] C. J. Bates, A. Bailey, H. van der Berg, F. van Schaik, C. Coudray, A. Faviet, R. Farré, A. Frigola, H. Heseker, G. Maiani, A. Ferro-Luzzi, K. Pietrzik, and D. I. Thurnham, *Intl. J. Vit. Nutr. Res.* **64,** 283 (1994).
[14] L. A. Pachla, D. L. Reynolds, and P. T. Kissinger, *J. Assoc. Anal. Chem.* **68,** 1 (1985).

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TABLE III
ANTIOXIDANT POWER OF TEAS AND WINES[a]

Sample	Amount	Antioxidant power (FRAP value) (μmol)
Fermented (black) teas, 1% infusion	200 ml	500–900
Semifermented teas, 1% infusion	200 ml	1000–1400
Nonfermented (green) teas, 1% infusion	200 ml	1600–2200
Red wines	150 ml	2900–3700
White wines	150 ml	380–520
Fresh orange juices (prepacked)	200 ml	500–600
Pure ascorbic acid (vitamin C)	1 g	11,364

[a] Range of values found in typical servings of teas and wines compared with orange juice and pure ascorbic acid.

permits the virtually simultaneous, specific measurement of ascorbic acid concentration and total reducing/antioxidant power (as FRAP) in one simple, rapid, automated test.[1,10] In FRASC, ascorbic acid in one of a pair of sample aliquots is selectively destroyed[15] by ascorbate oxidase [EC 1.10.3.3 (Sigma)]. Reagents are otherwise the same as in the FRAP assay. Reduction of the FRAP (FRASC) working reagent by ascorbic acid is complete within a few seconds of sample–reagent mixing. The 0- to 1-min post sample–reagent mixing absorbance change (at 593 nm) of a sample to which ascorbate oxidase (40 μl of a 4 U/ml solution added to a 100-μl sample) was added is subtracted from the absorbance change of a matching aliquot of sample to which water (40 μl added to a 100-μl sample), rather than ascorbate oxidase, was added; the difference is due specifically to ascorbic acid.[10,15] In FRASC, the 0- to 4-min absorbance change of the aliquot diluted in water is due to the combined reductive activity of all the reacting antioxidants present in the sample, i.e., the "total antioxidant capacity," or ferric reducing/antioxidant power (FRAP) value.[2] The 0- to 4-min and paired 0- to 1-min absorbance changes are translated into μM of FRAP and ascorbic acid, respectively, by comparison with those of standard solutions of Fe(II) or ascorbic acid of the appropriate molar concentration. It must be remembered that if Fe(II) standards are used for the calculation of ascorbic acid concentration, ascorbic acid has a stoichiometric factor of 2.0 in the FRAP assay; i.e. 1000 μM Fe(II) is equivalent to 1000 μM of FRAP but to only 500 μM of ascorbic acid. Similarly, if ascorbic acid standards are used for the calculation of FRAP values, these

[15] I. F. F. Benzie, *Clin. Biochem.* **29,** 111 (1996).

values are double the ascorbic acid concentration; i.e., 1000 μM ascorbic acid is equivalent to 2000 μM FRAP.

By monitoring the 0- to 4-min absorbance change of paired aliquots of water- and ascorbate oxidase-treated samples run in parallel on a Cobas Fara centrifugal analyzer and using the automated FRAP assay program detailed in Table I, all necessary data are gathered to obtain FRAP values and ascorbic acid concentrations of up to 13 pairs of test samples in one run. The concept of FRASC is represented in Fig. 5.

Calculation of Results

Using water-diluted samples,

$$\text{FRAP}\ (\mu M)\ \text{value} = \frac{\text{0- to 4-min}\ \Delta A_{593\,\text{nm}}\ \text{test sample}}{\text{0- to 4-min}\ \Delta A_{593\,\text{nm}}\ \text{standard}} \times [\text{FRAP}]_{\text{std}}\ (\mu M) \quad (2)$$

Using paired water (−ao)- and ascorbate oxidase-diluted (+ao) samples, the ascorbic acid concentration is calculated as follows:

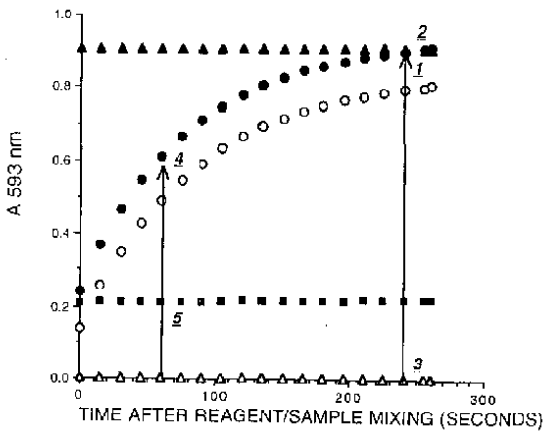

FIG. 5. Measuring concept of FRASC showing the absorbance change due to Fe(III)-TPTZ reduction by antioxidants in the sample. Calculation of the FRAP value is done by taking the 0- to 4-min ΔA for test sample (●, 1) and relating it to the 0- to 4-min ΔA for the Fe(II) standard (▲, 2), with a reagent blank correction (△, 3) for both. Calculation of ascorbic acid results is by subtracting the 0- to 1-min ΔA reading of the ascorbate oxidase-treated test sample (○) from the matching water-treated sample (●, 4); this signal is then related to that given by a standard solution of Fe(II) (▲) (or ascorbic acid, ■, 5) of appropriate concentration. Reproduced with permission from I. F. F. Benzie and J. J. Strain, *Redox Rep.* **3,** 233 (1997).

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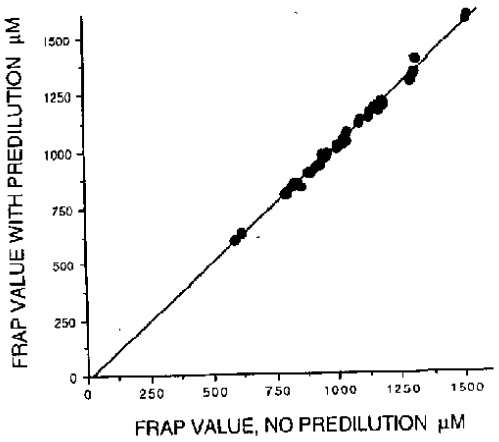

FIG. 6. Relationship between measured FRAP values on EDTA plasma with no predilution step and after predilution of 100 μl plasma with 40 μl water. Excellent agreement was seen ($r = 0.998$; $y = 1.03x - 23$), showing no net loss or gain of reductive activity with dilution. Reproduced with permission from I. F. F. Benzie and J. J. Strain, *Redox Rep.* **3**, 233 (1997).

$$\text{0- to 1-min ascorbic acid related } \Delta A_{593\,nm} = (\text{0- to 1-min } \Delta A_{593\,nm} \text{ sample } - \text{ao}) - (\text{0- to 1-min } \Delta A_{593\,nm} \text{ sample } + \text{ao}) \quad (3)$$

$$\text{Ascorbic acid concentration } (\mu M) = \frac{\text{0- to 1-min ascorbic acid related } \Delta A_{593\,nm} \text{ of test sample}}{\text{0- to 1-min ascorbic acid related } \Delta A_{593\,nm} \text{ of standard}} \times [\text{ascorbic acid}]_{std} \quad (4)$$

Data presented in Fig. 6 show FRAP values of 25 EDTA plasma samples measured with and without predilution in water (100-μl sample plus 40 μl of water). All samples were measured twice, in separate runs. FRAP values (μM) were obtained with reference to a Fe(II) standard solution run in

TABLE IV
FRAP VALUES AND ASCORBIC ACID CONCENTRATIONS (MEAN; MEDIAN; SD), USING FRASC, OF FRESH FASTING EDTA PLASMA FROM HEALTHY SUBJECTS[a]

Parameter	All (n = 130)	Men (n = 66)	Women (n = 64)
Age (years)	43; 43; 16.4	42; 42; 16.3	43; 44; 16.6
FRAP (μM)	1018; 1004; 198	1086; 1077; 189	948; 927; 183
Ascorbic acid (μM)	51; 48; 17.9	49; 48; 13.8	52; 50; 21.3

[a] Reproduced with permission from I. F. F. Benzie and J. J. Strain, *Redox Rep.* **3**, 233 (1997).

parallel and treated identically to the samples in each case, i.e., prediluted or nonprediluted in water as appropriate. Results obtained by the original FRAP assay (no predilution) and by FRASC show very good agreement ($r = 0.998$; $p < 0.0001$); mean (SD) FRAP values of the neat and prediluted samples were 998 (217) and 1010 (225) μM.

Precision of FRASC is good: within- and between-run CVs are, respectively, <1 and <3% at 900 and 1800 μM for FRAP values; for ascorbic acid, within- and between-run CVs are <5% at 25, 50, 100, and 440 μM.

Ferric reducing/antioxidant power values and ascorbic acid concentrations were measured,[10] using FRASC, in fresh EDTA plasma samples from 130 apparently healthy, fasting adults (66 men, 64 women) aged 21–74 years from whom informed consent had been obtained. Results are shown in Table IV.

Conclusions and Clinical Applications

The FRAP assay, in both its original[2] and modified, as FRASC,[10] versions, is robust, sensitive, simple, and speedy and will facilitate experimental and clinical studies investigating the relationship among antioxidant status, dietary habits, and risk of disease.[16–18] Measurement of the total antioxidant power of fresh biological fluids, such as blood plasma, can be measured directly, the antioxidant content of various dietary agents can be measured objectively and reproducibly, and their potential for improving the antioxidant status of the body investigated and compared. The FRAP assay is also sensitive and analytically precise enough to be used in assessing the bioavailability of antioxidants in dietary agents, to help monitor longitudinal changes in antioxidant status associated with an increased intake of dietary antioxidants, and to investigate the effects of disease on antioxidant status.

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